PB84-123801

Augmentation of Murine Natural Killer Cell Activity by Manganese Chloride

(U.S.) Health Effects Research Lab.
Research Triangle Park, NC

1983

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TECHNICAL REPORT DATA

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1. REPORT NO.	2.	3. R
EPA-600/J-83-121		PB84-123801

4. TITLE AND SUBTITLE	5. REPORT DATE
Augmentation of Murine Natural Killer Cell Activity by Manganese Chloride (Journal Version)	1983
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
R. Rogers, R. Garner, M. Riddle, R. Luebke, and R. Smialowicz	
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
Immunobiology Section/Cellular Biophysics/Experimental Health Effects Research Laboratory US Environmental Protection Agency Research Triangle Park, NC 27711	ACSL1A
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Office of Research & Development Health Effects Research Laboratory US Environmental Protection Agency Research Triangle Park, NC 27711	14. SPONSORING AGENCY CODE EPA-600/11

15. SUPPLEMENTARY NOTES

REF: Toxicology and Pharmacology, 70:7-17, 1983

16. ABSTRACT

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17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
Release to Public	Unclassified	12
	20. SECURITY CLASS (This page) Unclassified	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS

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NATIONAL TECHNICAL INFORMATION SERVICE
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EPA-600/J-83-121
JOURNAL ARTICLE

TOXICOLOGY AND APPLIED PHARMACOLOGY 70, 7–17 (1983)

Augmentation of Murine Natural Killer Cell Activity by Manganese Chloride[1]

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Received September 15, 1982; accepted April 16, 1983

Augmentation of Murine Natural Killer Activity by Manganese Chloride. Rogers, R. R., Garner, R. J., Riddle, M. M., Luebke, R. W., and Smialowicz, R. J. (1983). *Toxicol. Appl. Pharmacol.* **70**, 7–17. Natural killer (NK) cell activity of spleen cells from male CBA/J mice was augmented by a single parenteral injection of $MnCl_2$ administered 1 day prior to testing by *in vitro* and *in vivo* isotope release assays. Increased cytotoxic activity was observed *in vitro* against both NK-sensitive (YAC-1) and NK-resistant (EL-4) target cells. NK activity was also enhanced in normally low (A/J) and intermediately (C57Bl/6J) reactive strains of mice. Augmentation of NK activity by $MnCl_2$ was accompanied by an increase in circulating interferon (IFN) levels.

Concern for environmental exposures to heavy metals has led to an increased interest in the biological effects of these elements. Manganese is a ubiquitous trace metal to which elevated exposures are afforded by numerous mining and industrial processes. Profound biological effects have been observed following manganese exposure. Best known is the syndrome of neurotoxicity comparable to Parkinson's Disease (Chandra *et al.*, 1974). Available information suggests that this toxic effect is probably largely the consequence of Mn-altered function of enzymes associated with the synthesis and catabolism of certain neurochemicals (Deskin *et al.*, 1980; Donaldson *et al.*, 1980). Manganese has been reported to exert an influence on metabolic processes of certain cells by stimulating glycolysis and inhibiting lipolysis (Baquer *et al.*, 1975; Schimmel, 1978; Rennie and Upton, 1981). The toxicological significance of these effects has not been established. Other rather unique effects of manganese exposure have been noted in a few studies of carcinogenicity. For example, manganese has been shown to inhibit carcinogenesis (Sunderman *et al.*, 1976; Sunderman, 1979) and cell transformation (Costa *et al.*, 1981) by certain other metallic and nonmetallic compounds. Further, addition of manganese has been demonstrated to increase the potency of an anti-Meth A lymphosarcoma vaccine (McCollester, 1982). The molecular mechanism(s) of these effects have not yet been elucidated.

Several investigators have reported rather diverse effects of metals and metal compounds on both humoral and cell-mediated immune function. Frequently the extent or even direction of the observed effect depends not only on the metal, but also on its chemical form

[1] This report has been reviewed by the Office of Research and Development, EPA, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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0041-008X/83 $3.00
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or the method of exposure (Treagan, 1975; Koller, 1979; Lawrence, 1981). The investigation reported here was based on the earlier reports of manganese inhibition of carcinogenesis (Sunderman *et al.*, 1976; Sunderman, 1979) and on the recent observation in this laboratory that the cytotoxic activity of natural killer (NK) cells is particularly susceptible to suppression by the metal salt $NiCl_2$ (unpublished data). NK cells are lymphocytes which, without prior sensitization, are cytotoxic for certain tumor cells (Herberman *et al.*, 1979; Roder *et al.*, 1979). Consequently NK cells have been suggested to play a role in immune surveillance against newly arising tumor cells (Herberman and Holden, 1979; Herberman and Ortaldo, 1981; Herberman, 1981).

This investigation was conducted to determine the influence of manganese, administered as $MnCl_2$, on the activity of NK cells. The role that manganese modulation of cytotoxic activities may play in the manifestation of certain neoplasias is discussed.

METHODS

Animals. Male CBA/J and A/J mice and female C57Bl/6J mice (Jackson Laboratories, Bar Harbor, Maine) received at 8 weeks of age were maintained in plastic cages and provided feed (Wayne Lab Blox) and water *ad libitum* for at least 1 week prior to experimental treatment.

Metal treatment. A stock solution of manganous chloride ($MnCl_2 \cdot 4H_2O$, Fisher Scientific Co., Fair Lawn, N.J.) was prepared in sterile, nonpyrogenic, normal (0.9%) saline solution (Abbott Laboratories, North Chicago, Ill.), hereafter referred to simply as saline, and the manganese concentration was determined by neutron activation analysis (Dr. J. N. Weaver, North Carolina State University, Raleigh, N.C.). Shortly before use, aliquots of the stock solution were diluted to the desired concentration in saline buffered with 25 mM 2-*N*-hydroxyethyl-piperazine-*N'*-2-ethanesulfonic acid (Hepes, Grand Island Biological Co., Grand Island, N.Y.), hereafter referred to as Hepes–saline. Mice were given a single intramuscular (im) injection in the thigh, the route of administration used by Sunderman (1979) and Sunderman *et al.* (1976). Preliminary investigations demonstrated that equivalent effects were also obtained by sc or ip injection. Manganese was administered on a body weight basis with injection volumes ranging from 0.10 to 0.15 ml. Unless otherwise specified, CBA/J and A/J mice were given 80 μg $MnCl_2$/g body weight and C57Bl/6J were given 40 μg/g body weight 1 day prior to evaluation of NK activity. Control mice were injected with equivalent volumes of Hepes–saline.

Solutions of nickel chloride (Alfa Products, Danvers, Mass.) were prepared, diluted, and administered in a manner identical to that described above for $MnCl_2$. CBA/J mice were injected with 18.3 μg $NiCl_2$/g body weight, a treatment observed in previous investigations to suppress NK cell activity in CBA/J mice. Treatments which included both Mn and Ni in a single injection were administered in volumes also ranging from 0.10 to 0.15 ml. Sequential treatment with both metals was accomplished by injecting one of the two different metal solutions on each of 2 consecutive days, followed by measurement of NK activity on the following day.

Interferon inducers. Polyinosinic:polycytidylic acid (poly I:C, Grand Island Biological Co.) and tilorone hydrochloride (kindly provided by J. M. Cretsos, Merrel Dow Pharmaceuticals, Inc., Cincinnati, Ohio) were each dissolved in saline. Mice were treated with 0.1 mg poly I:C per animal, injected iv 18 hr prior to testing, or with 2 mg tilorone given by oral gavage 24 hr prior to testing.

Anti-interferon treatment. Saline and $MnCl_2$ treated C57Bl/6J mice were injected with rabbit anti-mouse interferon-α,β (Enzo Biochem, Inc., New York, N.Y.). Anti-interferon serum (2.8×10^4 neutralizing units/ml, NIH reference) was diluted in RPMI-1640 and mice were injected iv with 1000 neutralizing units 1 hr before and 500 neutralizing units 7 hr after treatment with Hepes–saline or 40 μg/g $MnCl_2$. Serum controls were injected with RPMI-diluted normal rabbit serum at the same volumes and times. Additional controls were treated only with Hepes–saline or 40 μg/g $MnCl_2$. The *in vitro* NK assay was performed 24 hr following metal treatment.

Medium. All spleen cell and tumor cell suspensions were prepared, labeled, and cultured in RPMI-1640 containing 2 mM glutamine, 25 mM Hepes, 10% heat-inactivated fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin (Grand Island Biological Co.). All cultures and incubations were maintained at 37°C with 5% CO_2.

Effector cells. Mice were killed by cervical dislocation, and spleens were aseptically removed and placed in complete RPMI. Cell suspensions were prepared by gently teasing the spleen, then disrupting cell clumps by repeated pipeting. Clumps of cells and debris were allowed to settle, and suspended cells were transferred and pelleted by centrifugation for 5 min at 250*g*. Erythrocytes were lysed by addition of 0.83% NH_4Cl. Spleen cells were then washed, counted with a hemacytometer, and resuspended to 2×10^7 viable cells/ml. Viability was determined by trypan blue dye exclusion.

Target cells. YAC-1 tumor cells (obtained from Dr. Ann Burger, Duke University, Durham, N.C.) were maintained in culture in complete RPMI. EL-4 cells (obtained from Dr. Dolph Adams, Duke University) were stored at −90°C and cultured in complete RPMI for at least 1 week

prior to use as targets. YAC-1 or EL-4 cells were labeled for use in the *in vitro* NK assay by incubating for 1 hr in complete RPMI with $Na_2{}^{51}CrO_4$ (Specific Activity: 200–500 Ci/g, New England Nuclear, Boston, Mass.) added at a concentration of 200 μCi ^{51}Cr/3 × 10^6 cells/ml. Labeled cells were washed three times to remove unbound ^{51}Cr and resuspended to 2 × 10^5 cells/ml.

NK activity. Natural killer cell activity was examined *in vitro* by a modification of the assay described by Kiessling *et al.* (1977). ^{51}Cr-labeled target cells (2 × 10^4/well) were cocultured in triplicate with effector cells for 4 hr in a volume of 200 μl in round-bottomed microtiter plates (Flow Laboratories, Inc., McClean, Va.) at effector:target cell ratios (E:T) of 25:1, 50:1, and 100:1. After incubation, cells were pelleted by centrifugation (5 min at 250*g*) and the supernatant fraction was collected with the Titertek supernatant collection system (Flow Labs, Inc.). Radioactivity in the supernatant fraction was measured in a gamma scintillation spectrometer (Model C5986, Packard Instrument Co., Downers Grove, Ill.). Total incorporated ^{51}Cr activity was determined by direct addition of 2 × 10^4 washed, labeled target cells to replicate counting tubes. Maximum releasable radioactivity, determined from the supernatant fraction of cultured wells containing labeled target cells in the presence of 0.25% Triton X-100 (New England Nuclear), was typically 65–75% of the total incorporated radioactivity. Spontaneous release, as determined from cultures containing only labeled target cells, was typically 5–10% of the maximum releasable ^{51}Cr activity. The replicate mean was used to calculate percentage cytotoxicity according to the equation:

$$\% = \frac{\text{Experimental} - \text{Spontaneous}}{\text{Maximum} - \text{Spontaneous}} \times 100$$

In vivo NK activity was evaluated according to the procedure described by Riccardi *et al.* (1980). Briefly, YAC-1 tumor cells were labeled with ^{125}I-5-iodo-2′deoxyuridine (New England Nuclear Corp., Boston, Mass.) in the presence of 5-fluoro-2′-deoxyuridine, washed, and resuspended to 2 × 10^6 cells/ml in unsupplemented RPMI-1640. One million labeled cells were then injected into the tail vein of each mouse. Mice were killed 4 hr later by cervical dislocation; their spleens and lungs were removed and placed in tubes; organ radioactivity was measured. This method of measuring NK activity has been validated by demonstrating the correlation between NK activity measured *in vitro* and the *in vivo* clearance of labeled tumor cells. A correlation has been observed for several variables such as strain differences in NK activity, tumor cell susceptibility to NK lysis, and chemical modulation of NK activity (Riccardi *et al.*, 1980; Herberman, 1981). Results are expressed as percentage recovery (geometric mean) or as ln-percentage recovery of the injected radioactivity.

Interferon titer. Plasma samples were obtained from mice which had been treated the previous day with either Hepes–saline, $MnCl_2$, poly I:C, or tilorone. Immediately before mice were killed for *in vitro* NK assay, they were injected ip with 100 units of heparin and 10 min later placed under mild ether anesthesia. Blood was drawn from the retro-orbital sinus, and plasma samples were frozen and shipped to Meloy Laboratories (Springfield, Va.) for analysis of interferon titer (α, β, and γ) by assay of inhibition of the cytopathic effect of Vesicular Stomatitis Virus (VSV) upon murine L929 cells.

Statistical analysis. Determination of Mn dose dependence was accomplished by linear regression analysis. Treatment mean values of percentage cytotoxicity, percentage *in vivo* ^{51}Cr recovery (ln transformed), and interferon titer (log transformed) were compared by Dunnett's *t* test.

RESULTS

Spleen cells from male CBA/J mice, 24 hr after treatment with 40, 80, or 120 μg/g $MnCl_2$, exhibited a dose related increase ($p < 0.01$) in NK activity as evidenced by *in vitro* cytolytic release of ^{51}Cr from NK-sensitive YAC-1 target tumor cells (Fig. 1). A study of the time course of the effect of Mn treatment (80 μg/g) showed that NK activity was sustained at significantly enhanced levels for at least 3 days before returning to normal (Fig. 2). Manganese-induced enhancement of NK activity was reproduced in numerous experiments. This augmentation of NK activity was apparently due to an influence, either direct or indirect, upon NK cells rather than the result of selective elimination of other cell types from the spleens of Mn-treated mice. Statistical analyses, collective (>600 mice) and of individual experiments, unequivocally demonstrated no correlation between total spleen cellularity and the influence of Mn on NK activity (data not shown).

Table 1 shows that $MnCl_2$ also enhanced the normally lower cytotoxic activities of spleen cells from C57Bl/6J and A/J mice against YAC-1 cells. Spleen cells from Mn-treated CBA/J mice displayed enhanced cytotoxicity against relatively NK-resistant EL-4 tumor cells (Table 1). Older CBA/J mice, which in our hands exhibit normal levels of NK activity, also showed enhanced splenic NK activity 24 hr following $MnCl_2$ injection (Table 1).

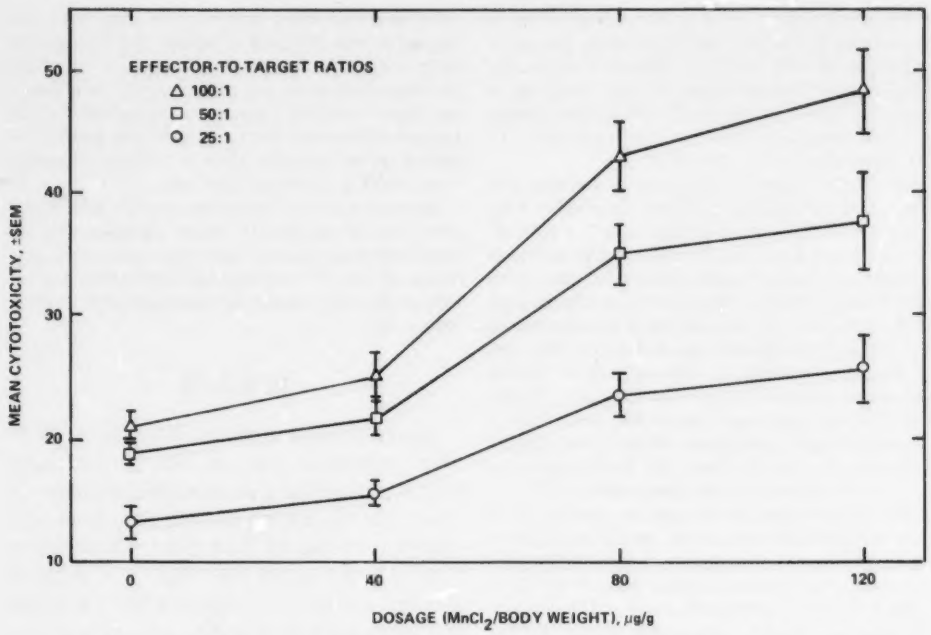

FIG. 1. Influence of manganese on NK cell activity in 11-week-old male CBA/J mice. Spleen cells were coincubated for 4 hr with YAC-1 tumor cells at effector:target cell ratios (E:T) of 25:1 (○), 50:1 (□), and 100:1 (△). Suspensions of cells from Mn-treated mice exhibited a dose-dependent ($p < 0.01$) increase in NK activity. Six (6) mice per treatment group.

The effect of treatment with both $MnCl_2$ (80 μg/g) and $NiCl_2$ (18.3 μg/g), either administered separately or in a single im injection, is shown in Table 2. A single injection of $NiCl_2$ significantly suppressed NK activity compared with Hepes–saline injected controls (Experiment 1, Table 2). This suppression was preempted by the concurrent administration of $MnCl_2$, which resulted in NK enhancement comparable to that obtained by $MnCl_2$ injection alone. $MnCl_2$ administered prior but not subsequent to $NiCl_2$ treatment also resulted in enhanced NK activity (Experiment 2, Table 2).

The influence of Mn on *in vivo* NK activity, examined by measuring the clearance of radiolabeled YAC-1 cells from the lungs and spleen of CBA/J mice treated 24 hr earlier with Hepes–saline or $MnCl_2$ (80 μg/g), is shown in Fig. 3 and Table 3. The radiolabeled cells are initially predominantly localized in the lungs, from which they are rapidly cleared. Radioactivity recovered from the lungs is reduced from about 20% at 30 min after injection to less than 1% after 4 hr. Enhanced clearance, shown by a decrease in percentage radioactivity recovered, was observed in the lungs of Mn-treated mice at 1, 2, and 4 hr, and in their spleens at 4 hr. Table 3 shows that $MnCl_2$ also augmented the *in vivo* NK activity in low responder A/J mice. $MnCl_2$ also enhanced *in vivo* NK activity in older CBA/J mice which, again, in our hands display normal NK cell activity.

It should be pointed out that the data presented in Tables 1 and 3 do not support the frequently described diminished NK activity in older mice (Gorelik and Herberman, 1981; Luini *et al.*, 1981; Riccardi *et al.*, 1981). Furthermore, the results of additional experiments

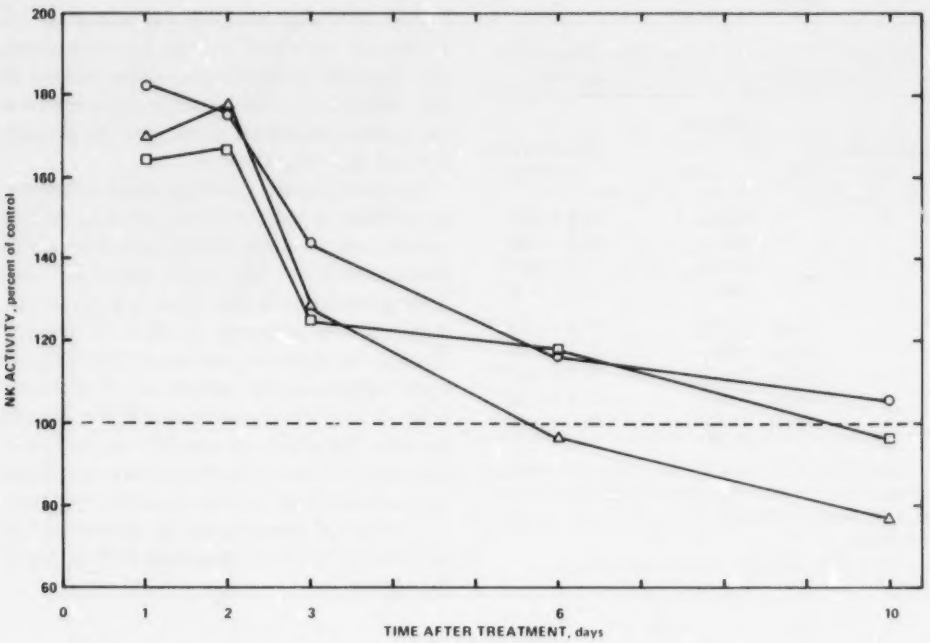

FIG. 2. Time course of the effect of 80 μg/g $MnCl_2$ on *in vitro* NK activity observed at effector:target ratios of 25:1 (○), 50:1 (□), and 100:1 (△). For Days 1 thru 3, $p < 0.01$ for all E:T's except Day 3—100:1 ($p < 0.05$). Six (6) male CBA/J mice, 11 weeks of age, per treatment group.

intended to specifically compare the NK activity against YAC-1 targets in old and young mice also failed to demonstrate reduced activity in older CBA/J or C57Bl/6J mice (data not shown).

Analysis of plasma from mice treated 24 hr prior with 80 μg/g $MnCl_2$ demonstrated elevated interferon titers (Table 4). The interferon titers of Mn-treated mice were lower than levels observed for mice treated with the

TABLE 1

STIMULATION OF *IN VITRO* NK CELL ACTIVITY BY MANGANESE

Cell type				% Cytotoxicity ± SEM	
Effector source	Age (weeks)	No. per treatment	Target[a]	Control	$MnCl_2$[b]
CBA/J	11	6	YAC-1	27.6 ± 2.0	45.3 ± 2.0*
CBA/J	11	10	EL-4	1.3 ± 0.5	10.6 ± 1.1*
A/J	9	8	YAC-1	6.5 ± 1.7	19.4 ± 2.5*
CBA/J	19	12	YAC-1	28.6 ± 1.1	47.8 ± 2.0*
C57Bl/6J	11	6	YAC-1	9.6 ± 0.52	26.0 ± 1.60*

[a] Effector:target ratio = 50:1.
[b] CBA/J, A/J—80 μg/g; C57Bl/6J—40 μg/g.
* $p < 0.01$, Dunnett's t test.

TABLE 2

IN VITRO NK ACTIVITY[a] FOLLOWING TREATMENT WITH 80 μg/g $MnCl_2$ AND/OR 18.3 μg/g $NiCl_2$

Experiment No.	Treatment Day 1	Treatment Day 2	% Cytotoxicity ± SEM
1		Saline	12.3 ± 0.81
		$MnCl_2$	21.9 ± 1.67*
		$MnCl_2$ + $NiCl_2$	23.4 ± 0.83*
		$NiCl_2$	4.8 ± 1.08*
2	Saline	Saline	24.8 ± 1.05
	Saline	$NiCl_2$	20.4 ± 2.41
	Saline	$MnCl_2$	39.9 ± 2.85**
	$NiCl_2$	$MnCl_2$	19.8 ± 3.32
	$MnCl_2$	$NiCl_2$	46.0 ± 1.69**

[a] Male CBA/J, 11 weeks of age, 6 mice per treatment group; YAC-1 target cells, effector:target ratio = 50:1, separate assays performed 24 hr after single or second treatment.

* $p < 0.01$, Dunnett's t test, Experiment 1.

** $p < 0.01$, Dunnett's t test, Experiment 2.

known IFN inducers poly I:C and tilorone. Treatment with $MnCl_2$, poly I:C, or tilorone also resulted in significant enhancement of NK cell activity, with tilorone inducing both the highest interferon levels and the greatest NK cell activity (Table 4).

To determine the extent to which interferon is involved in the Mn augmentation of NK activity, mice were pretreated with rabbit anti-mouse interferon. The results, shown in Table 5, demonstrate that interferon is a major mediator of the observed increase in activity. Though the data are confounded by a significant suppression by normal serum of the activity of both Hepes–saline and $MnCl_2$-treated animals, the ability of anti-IFN to reduce or eliminate the Mn-induced increase in NK activity against YAC-1 cells is readily apparent. The observed suppression by normal serum may be due to stress associated with giving iv injections.

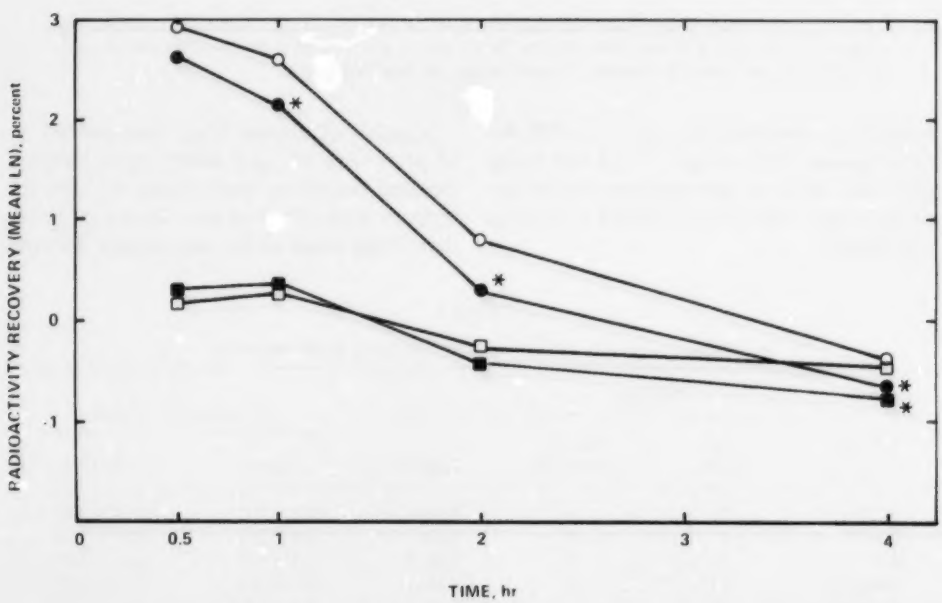

FIG. 3. The effect of 80 μg/g $MnCl_2$ on the kinetics of *in vivo* NK activity in spleen and lungs. A significant increase ($p < 0.01$) in the clearance of radiolabeled YAC-1 tumor cells from the spleens (■—$MnCl_2$ vs □—saline) of Mn-treated CBA/J mice (11 weeks) was observed after 4 hr. An increase ($p < 0.01$) was detected in clearance from lungs (●—$MnCl_2$ vs ○—saline) after 1 hr. Ten (10) mice per treatment group.

TABLE 3

STIMULATION OF *IN VIVO* NK CELL ACTIVITY BY $MnCl_2$

Strain	Age (weeks)	Treatment	No. tested	Mean percentage *in vivo* recovery of radioactivity at 4 hr	
				Spleen	Lungs
CBA/J	10	Saline	10	0.59 (0.49–0.66)[a]	0.66 (0.51–0.93)
		$MnCl_2$[b]	10	0.43 (0.32–0.54)*	0.51 (0.40–0.60)*
CBA/J	22–24	Saline	9	0.55 (0.42–0.81)	0.71 (0.30–1.41)
		$MnCl_2$[b]	7	0.42 (0.27–0.63)	0.45 (0.32–0.62)*
A/J	10	Saline	12	0.48 (0.28–0.72)	2.51 (1.51–5.89)
		$MnCl_2$[b]	12	0.37 (0.25–0.52)*	0.85 (0.45–2.24)*

[a] Geometric mean (range of values).
[b] Dose—80 μg/g.
* $p < 0.05$, Dunnett's t test of log-transformed percentage recovery.

DISCUSSION

Results of this investigation clearly indicate that manganese, administered as $MnCl_2$, is capable of enhancing murine NK activity against certain tumor cells. Two possible explanations for the augmentation of NK activity are: (1) increased lytic efficiency, due to a positive influence of Mn^{2+} on suggested cytolytic mechanisms such as lysosomal enzymes (Roder *et al.*, 1980; Sanderson, 1981; Herberman and Holden, 1979) or the divalent cation (Mg^{2+} and Ca^{2+}) dependent NK lytic cycle described for both humans (Hiserodt *et al.*, 1982) and mice (Quan *et al.*, 1982); or (2) stimulated synthesis or release of an immunoregulatory compound known to enhance NK activity. The possibility of the former cannot be excluded, even though Mn^{2+} has been shown to inhibit a similar lytic event mediated by cytolytic T-lymphocytes (Gately and Martz, 1981). The occurrence of the latter, an immunoregulatory compound, was confirmed by the presence of elevated levels of interferon and by the suppression of Mn-enhanced NK activity by treatment with anti-interferon serum. Documentation of the rather broad scope of immunomodulating effects of interferon(s) is vast (for a review and references see De Maeyer and De Maeyer-Guignard, 1981), including numerous reports of the activation of existing NK cells or the recruitment and activation of pre-NK cells.

The observed augmentation of NK activity may not be the sole cytotoxic activity altered by $MnCl_2$ treatment, and interferon may not be the exclusive mediator of that effect. The 4-hr cytotoxicity assay employed here with lymphoma targets is generally regarded as a demonstration of activity mediated primarily by NK cells (Tagliabue *et al.*, 1979; Wiltrout *et al.*, 1982). By accepting that NK activity is the primary observation, augmentation of ^{51}Cr release from both YAC-1 and EL-4 points out the heterogeneity of NK cells and suggests the involvement of at least one other regulatory

TABLE 4

IN VITRO NK ACTIVITY[a] AND PLASMA INTERFERON

Treatment	% Cytotoxicity ± SEM	Interferon titer (IU)
Saline	12.6 ± 1.6	23 (18–72)[b]
$MnCl_2$	30.8 ± 2.3*	76 (18–288)[c]
Poly I:C	32.2 ± 1.4*	162 (72–288)[c]
Tilorone	40.5 ± 3.9*	290 (144–1200)[c]

[a] Male CBA/J, 11 weeks of age, 6 mice per treatment group; YAC-1 target cells, effector:target ratio = 50:1.
[b] Geometric mean (range of values).
[c] $p < 0.01$, Dunnett's t test of log-transformed titers.
* $p < 0.01$, Dunnett's t test.

TABLE 5

REQUIREMENT FOR INTERFERON FOR Mn-STIMULATED NK ACTIVITY AGAINST YAC-1

Treatment[a]	% Cytotoxicity[b] ± SEM	Treatment	% Cytotoxicity ± SEM
Saline	12.5 ± 0.4	$MnCl_2$	28.9 ± 1.5*
Saline + normal serum	9.1 ± 0.8**	$MnCl_2$ + normal serum	23.7 ± 1.4*
Saline + anti-IFN	11.4 ± 0.8	$MnCl_2$ + anti-IFN	8.6 ± 0.4*,**

[a] Female C57Bl/6J mice, 8 per treatment group, 40 μg/g $MnCl_2$.
[b] Effector:target ratio = 50:1.
* $p < 0.01$, Dunnett's t test vs appropriate saline group.
** $p < 0.01$, Dunnett's t test vs saline or $MnCl_2$ control group.

compound. Numerous attempts have been made to discriminate among the subsets of NK-mediating lymphocytes by virtue of differences in functional specificity and/or serological phenotype. From such efforts, it has been shown in C57Bl/6J mice that several subsets of NK cells are cytolytic for the NK-sensitive YAC-1 cells while fewer subsets are effective against NK-resistant EL-4 target cells which also happen in this instance to be syngeneic (Minato *et al.*, 1981). Further, while YAC-1 effector subsets are stimulated by interferon, the EL-4 effector subset, whose serological phenotype is basically like that of conventional alloreactive T-killer cells, is stimulated by interleukin-2 rather than interferon (Minato *et al.*, 1981). The involvement of still other potential NK regulators such as cyclic nucleotides (Roder and Klein, 1979) or prostaglandin E_2 (Koren and Leung, 1982) may be suggested from the previously reported ability of manganese to activate cyclic nucleotide phosphodiesterase (Rennie and Upton, 1981) or its ability to block tryglyceride lipase activation (Schimmel, 1978) in other cell types. However, at this time such discussion is highly speculative and beyond the scope of the present investigation.

The mechanism by which treatment with $MnCl_2$ induces the production of interferon is unclear. Sudo *et al.* (1981) reported that $CaCl_2$ enhanced the production of interferon by poly I:C stimulated human MG-63 cells. In experiments with control treatments of saline, Hepes-buffered saline, hypertonic saline, and unbuffered $MnCl_2$ solutions (data not shown), we confirmed that $MnCl_2$ was the solution component responsible for increased NK activity. Intermediate effects such as nonspecific inflammation, which occasionally occur following parenteral exposure, could also lead to the observed increase in interferon production (Djeu *et al.*, 1979; Neumann *et al.*, 1980). Efforts to determine if such effects occur following $MnCl_2$ treatment have been inconclusive. However, even if such intermediate effects are involved, the lack of NK enhancement by any of the other parenteral treatments employed here suggests that the resulting augmentation is Mn specific. If, in fact, $MnCl_2$ is capable of directly inducing interferon production, i.e., without dependence upon such intermediate cellular effects, it is a uniquely simple compound when compared to other known interferon inducers (Mayer, 1980). Such a simple compound could prove valuable in further elucidating the mechanism(s) of interferon production.

In this study we have observed a unique heavy metal-induced effect upon murine immune function. Manganese has previously been shown to reduce the incidence of tumor induction in rats (Sunderman *et al.*, 1976; Sunderman, 1979). That observation could conceivably include an augmented NK antitumor activity as reported here. However, conditions employed by Sunderman were such that it is unlikely that augmented NK activity would be responsible for more than a minor contribution to the observed "anticarcinoge-

nicity." The manganese treatment employed was at lower levels than those for which we observed significant NK augmentation. Nickel subsulfide was administered at highly neoplastic levels, possibly sufficient to overwhelm the anti-tumor surveillance of NK cells. Further, the reduced incidence of sarcomas observed by Sunderman (1979) was evident when manganese and nickel subsulfide were mixed and administered in a single im injection, but absent when administered in separate, contralateral injections. Such an apparently local rather than systemic effect seems inconsistent with either an immunological or enzymatic influence of Mn. However, evidence of some localization of NK regulation does exist (Hisano and Hanna, 1982). The inhibitory effects of manganese were tentatively attributed by Sunderman (1979) to competitive cellular compartmentalization. However, manganese was also reported to inhibit sarcoma induction by benzo(*a*)pyrene (Sunderman, 1979), in which case competitive metal ion phenomena would not apply. In an earlier report, Sunderman *et al.* (1974) suggested that nickel carcinogenesis might be inhibited by manganese through antagonism of previously reported nickel inhibition of RNA polymerase activity (Sunderman and Esfahani, 1968).

Yamane and Sakai (1973) demonstrated inhibition of the carcinogenicity of aminoazo dyes by manganese and other metals. This inhibition correlated with metal ion stimulation of certain detoxifying hepatic enzymes. In yet another investigation Mn dust was shown to inhibit *in vitro* neoplastic transformation of hamster embryo cells by crystalline Ni compounds (Costa *et al.*, 1981). Further, an obvious correlation was shown between cell transformation and phagocytosis of the Ni particles, which in turn was inhibited by either Mn dust or $MnCl_2$ without significant incorporation of Mn into the cell (Costa *et al.*, 1981). The diverse mechanisms operant in the above cases of Mn-inhibited neoplasia are evidence of the broad spectrum of biological effects of this heavy metal.

A conspicuous contrast exists between observed effects of Mn and Ni exposures on interferon production and NK activity. Treagan and Furst (1970) observed that $NiCl_2$ inhibited interferon production in cultured mouse L 929 cells, while in the present study (Table 2) reduced NK activity was observed following *in vivo* nickel treatment. The former observation included evidence that Ni inhibited interferon production by blocking interferon synthesis rather than its release. The contrasting effects of Mn and Ni on interferon production are certainly consistent with the hypothesis concerning RNA polymerase activity which was proposed by Sunderman *et al.* (1974), but the relevance has not been explicitly determined. Our investigation of the effect of concurrent and sequential treatments upon NK activity fails to support a suggestion that a common, reversible mechanism in interferon production exists for which these two metals might compete. Instead, the NK-enhancing effect of Mn was dominant in all cases except that in which Ni treatment preceded Mn treatment. There, the suppressed NK activity established by Ni could not be reversed by subsequent Mn treatment (Table 2).

Collectively, the observed effects of Mn and Ni on interferon production and NK activity suggest a potentially major effect of heavy metals which merits consideration in further studies of neoplastic transformation and metal toxicity. Even with the current paucity of data correlating cytotoxic activity with tumor induction, it seems reasonable to suggest, since such activity is their definitive function, that altered activity of cytotoxic cells such as NK can have significant influence on the proliferation of newly arising and slowly developing tumors.

ACKNOWLEDGMENT

We thank T. Wall for preparation of this manuscript.

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